chain immunoglobulin polypeptide. Support for a fusion polypeptide comprising a P-selectin glycoprotein ligand-1 polypeptide is found in the specification at page 5, line 7-9. Support for a fusion polypeptide comprising a heavy chain immunoglobulin polypeptide is found in the specification at page 5 lines 37-38. The specification recites "the antigenic fusion protein according to the invention is ... an immunoglobulin or part thereof". It would be well known to one of ordinary skill in the art that the heavy chain is part of an immunoglobulin (See pp 109-111 of Immunology Second Edition, by Janis Kuby, 1994, attached as Exhibit A). Applicant's assert that the phrases "comprising a P-selectin glycoprotein ligand-1 and "heavy chain immunoglobulin polypeptide" meets the written description requirement of § 112, first paragraph and requests that this rejection be withdrawn.

The Examiner rejected Claim 24 for lack of written description for the phrase "an extracellular portion of a P-selectin glycoprotein ligand-1". Applicants disagree. Claim 24 has been canceled, however new independent claim 29, and new dependent claims 30- 32 are directed to a fusion polypeptide comprising an extracellular portion of a P-selectin glycoprotein ligand-1, thus this rejection will be addressed with respect to new claims . The specification as filed provides support for a fusion polypeptide comprising an extracellular portion of a P-selectin glycoprotein ligand-1. Specifically, at page 8, lines 6-10 of the specification, Applicants describe the construction of PSGL1/mIgG_{2b}. "The muscin/immunoglobulin expression plasmid was constructed by fusing the PCR-amplified cDNA of *the extracellular part of PSGL-1* in frame with a BamH1 site, to the Fc part (hinge, CH2 and CH3) of mouse IgG_{2b} carried as an expression casette in CDM7." (Emphasis added). Thus, Applicant's assert that the phrase "extracellular portion of a P-selectin glycoprotein ligand-1" meets the written description requirement of § 112, first paragraph and requests that this rejection as it applies to new claims 29-32 be withdrawn.

The Examiner has rejected claim 25 for lack of written description for the phrase "a region of a heavy chain immunoglobulin polypeptide". Claim 25 has been canceled. Applicants request this rejection be withdrawn.

The Examiner has rejected claim 28 for lack of written description for the phrase "comprises more Galα1,3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1.". Applicants disagree. The specification as filed provides support for a fusion polypeptide comprising more Galα1,3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1.

Specifically, on page 12, lines 23-37, Applicants describe an experiment testing the capacity of Galα1,3Gal substituted PSGL1/mIgG_{2b} compared to non-Galα1,3Gal substituted PSGL1/mIgG_2 to remove Galα1,3Gal antibodies from human serum . The results of this experiments (see, FIG. 3) demonstrate that Galα1,3Gal substituted PSGL1/mIgG_{2b} has a higher absorption capacity for Galα1,3Gal antibodies than non-Galα1,3Gal substituted PSGL1/mIgG_{2b}. This is evident from the decrease in porcine endothelial cell cytotoxicity following absorption. It would be obvious to one skilled in the art that this higher absorption capacity of Galα1,3Gal substituted PSGL1/mIgG_{2b} for Galα1,3Gal antibodies was a direct result of the of the Galα1,3Gal substituted PSGL1/mIgG_{2b} having more Galα1,3Gal epitopes than the non-Galα1,3Gal substituted PSGL1/mIgG_{2b} (*i.e.*, wild type PSGL1). Thus, Applicant's assert that the phrase "more Galα1,3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1" meets the written description requirement of § 112, first paragraph and requests that this rejection as it applies to claims 28 and new claims 33 and 38 be withdrawn.

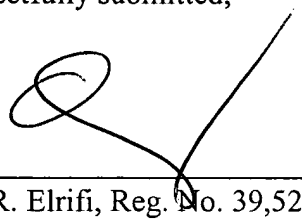
§ USC 112, First Paragraph Rejection, Enablement

The Examiner has rejected claims 21- 26, and 28 under 35 USC 112 first paragraph for lack of enablement. The Examiner asserts that the specification does not reasonably provide enablement for a dimerized fusion protein comprising at least a region of a PSGL-1 and at least a region of an immunoglobulin polypeptide because "the specification fails to disclose a definition for 'at least a region.'" (Office action, page 4 line 2). Applicants have amended independent claim 21 (from which 22-26 and 28 depend) to delete the phrase "at least a region". Therefore this rejection is now moot.

CONCLUSION

Applicants believe that the claims, as amended are in condition for allowance. If the Examiner has any questions, the Examiner is invites to contact the undersigned by telephone.

Respectfully submitted,



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Claim 21 was amend as follows:

21. A dimerized fusion polypeptide comprising a first polypeptide operably linked to a second polypeptide, wherein the first polypeptide:

(a) comprises [at least a region of] a P-selectin glycoprotein ligand-1; and

(b) is glycosylated by an α1,3 galactosyltransferase and

the second polypeptide comprises [at least a region of] immunoglobulin heavy chain polypeptide.

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EXHIBIT A

SECOND EDITION

IMMUNOLOGY

JANIS KUBY

Cover credits
Background: lymph node macrophage attached to an endothelial cell.

Inset: X-ray crystallography of a peptide bound to a human class II MHC molecule, DR1.
Courtesy of J. H. Brown, 1993, *Nature* 364:33.

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5

IMMUNOGLOBULINS: STRUCTURE AND FUNCTION

Immunoglobulins function as antibodies, the antigen-binding proteins that are present on the B-cell membrane and also are secreted by plasma cells. Secreted antibodies circulate in the blood and serve as the effectors of humoral immunity by searching out and neutralizing or eliminating antigens. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones depends on interaction of membrane antibody and antigen. All immunoglobulins share certain structural features, bind to antigen, and participate in a limited number of effector functions. This chapter focuses on how the primary, secondary, and tertiary structure of immunoglobulins contribute to both their specificity and their effector functions.

Basic Structure of Immunoglobulins

It has been known since the turn of the century that antibodies—the effector molecules of humoral immunity—reside in the serum. Identification of the serum-protein fraction containing antibodies was accomplished in a classic experiment by A. Tiselius and E. A. Kabat in 1939. They immunized rabbits with a protein antigen, ovalbumin (the albumin of egg whites), and then divided the immunized rabbits' serum into two aliquots. The first serum aliquot was separated by electrophoresis into four fractions: albumin and the alpha (α), beta (β), and gamma (γ) globulins. The second serum aliquot was reacted with antigen, so that antibody bound to the ovalbumin was

precipitated and could be removed; then the remaining serum proteins were electrophoresed. A comparison of the electrophoretic profiles of these two serum aliquots revealed that there was a significant drop in the γ-globulin peak in the aliquot that had been subjected to precipitation with antigen (Figure 5-1). Thus the γ-globulin fraction was identified as containing serum antibodies, which were called *immunoglobulins* to distinguish them from any other proteins that might be contained in the γ-globulin fraction.

In the 1950s and 1960s experiments by Rodney Porter and by Gerald Edelman elucidated the basic structure of the immunoglobulin (Ig) molecule. (These experiments were considered of such significance that the two investigators shared a Nobel prize in 1972.) Edelman's and Porter's experimental approaches were quite different. Porter cleaved the Ig molecule with enzymes to obtain fragments, whereas Edelman dissociated the molecule by reducing the interchain disulfide bonds. The results attained by these two approaches complemented each other and allowed the basic structure of the Ig molecule to be elucidated.

Using ultracentrifugation, both Porter and Edelman first separated the γ-globulin fraction of serum into a high-molecular-weight fraction with a sedimentation constant of 19S and a low-molecular-weight fraction with a sedimentation constant of 7S. They used the 7S fraction, containing a 150,000-MW γ-globulin designated as immunoglobulin G, or IgG, for their studies. Porter subjected IgG to brief digestion with the enzyme papain and separated the fragments. Although papain has general, nonspecific proteolytic activity and will eventually digest the entire IgG molecule, brief treatment cleaves only the most susceptible bonds. Papain digestion of IgG produced two identical fragments (each with a MW of 45,000) called *Fab* fragments because they retained their "antigen-binding" activity and one fragment (MW of 50,000) called the *Fc* fragments because it was found to crystallize during cold storage (Figure 5-2a). A similar experimental approach, but with the enzyme pepsin, was taken by Alfred Nisonoff. Brief pepsin digestion generated a single 100,000-MW fragment composed of two Fab-like fragments and designated $F(ab')_2$. Like the Fab fragments, the $F(ab')_2$ fragment was also able to visibly precipitate antigens. However, after pepsin digestion, the Fc fragment was not recovered because it had been digested into multiple fragments.

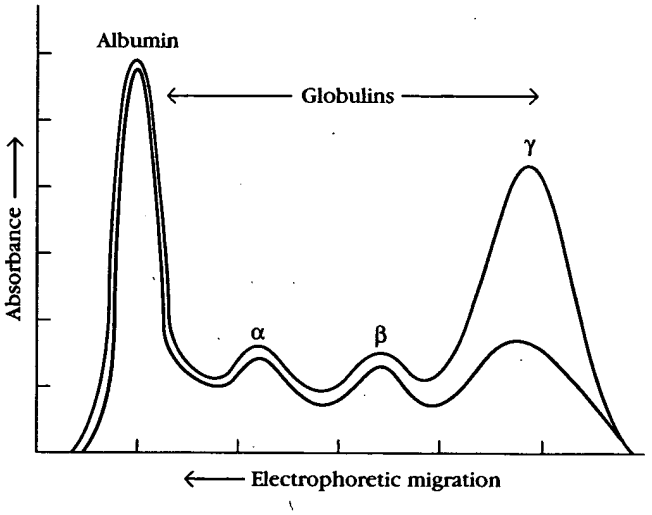

FIGURE 5-1 Experimental demonstration that antibodies are present in the γ-globulin fraction of serum proteins. After rabbits were immunized with ovalbumin (OVA), their antisera were pooled and electrophoresed, which separates the serum proteins based on electric charge. The purple line shows the electrophoretic pattern of untreated antiserum. The black line shows the pattern of antiserum that was incubated with OVA to remove anti-OVA antibody and then electrophoresed. [Adapted from A. Tiselius and E. A. Kabat, 1939, *J. Exp. Med.* **69**:119.]

The chain structure of IgG was first suggested by experiments of Edelman and his colleagues and later confirmed by Porter. Edelman reduced the disulfide bonds of IgG with mercaptoethanol and subjected the denatured protein to starch gel electrophoresis in 8 *M* urea, which reduces the intrachain as well as the interchain disulfide bonds and allows the molecule to unfold. Two electrophoretic bands were obtained, indicating that the IgG molecule contained more than one protein chain. Porter extended this study by doing a much milder mercaptoethanol reduction, so that only the interchain disulfide bonds were reduced. He then alkylated the exposed sulfhydryl groups with iodoacetamide to prevent random re-formation of the disulfide bonds and added an organic proprionic acid solvent to prevent aggregation. The sample was then chromatographed on a column that separates molecules on the basis of size (Figure 5-2b). This experiment revealed that the 150,000-MW IgG molecule was composed of two 50,000-MW polypeptide chains, designated as *heavy (H) chains*, and two 25,000-MW chains, designated as *light (L) chains*.

The remaining puzzle was to determine how the enzyme digestion products—Fab, $F(ab')_2$, and Fc—were related to the heavy-chain and light-chain reduction products. Porter answered this question by using antisera from goats that had been immunized with the Fab fragments and Fc fragments of rabbit IgG. He found that antibody to the Fab fragment could react with both the H and the L chains, whereas antibody to the Fc fragment reacted only with the H chain. These observations led to the conclusion that Fab consists of

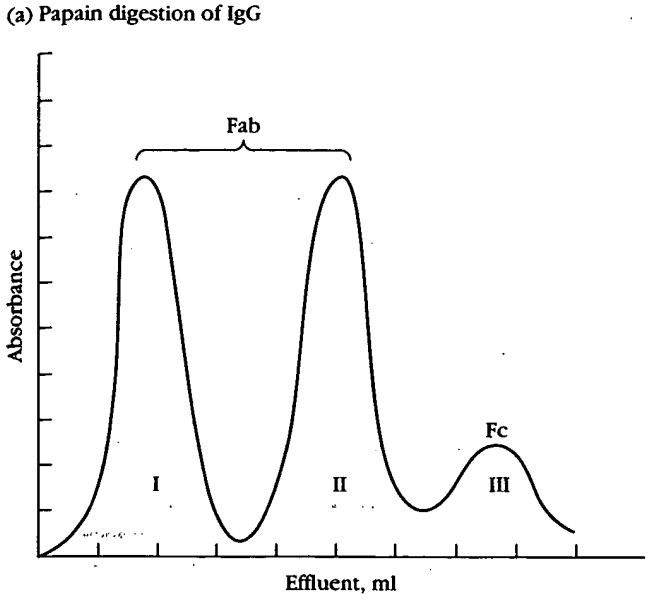

FIGURE 5-2 Separation of products resulting from enzymatic digestion or mild reduction of IgG. (a) Brief digestion of heterogeneous IgG with papain and separation of the digest on a carboxymethylcellulose column yields three peaks. Peaks I and II contain the 45,000-MW Fab fragments, and peak III contains the 50,000-MW Fc fragment. Digestion of homogeneous IgG yields a single Fab peak, which is the sum of peaks I and II. (b) Mild reduction of IgG and alkylation of the exposed sulfhydryl groups followed by gel filtration in proprionic acid yields two peaks. Peak I contains the 50,000-MW heavy chain, and peak II contains the 25,000-MW light chain. [Part (a) adapted from R. R. Porter, 1959, *Biochem. J.* **73**:119; part (b) adapted from J. B. Fleischman, 1962, *Arch. Biochem. Suppl.* **1**:1974.]

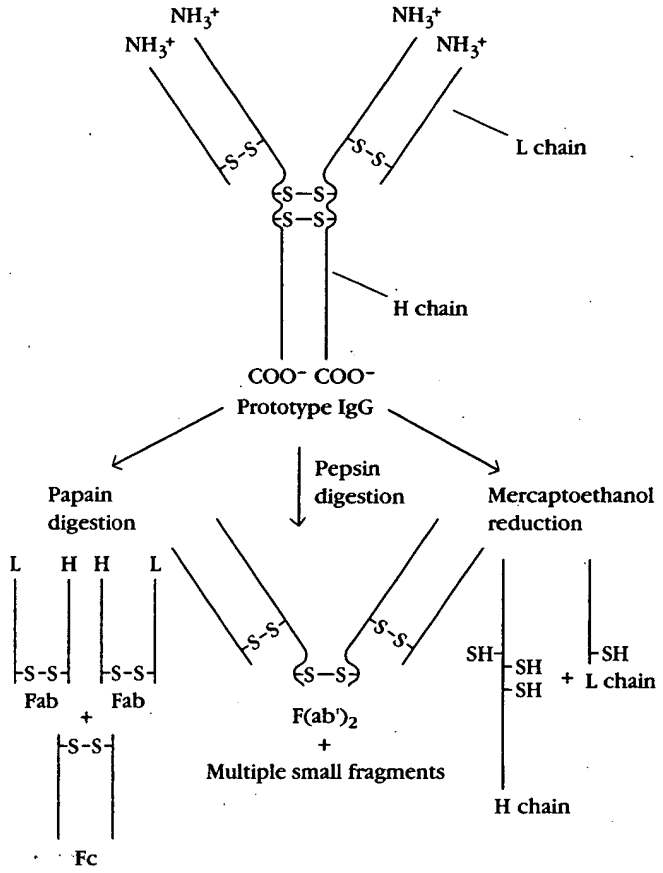

FIGURE 5-3 Prototype structure of IgG, proposed by Rodney Porter in 1962, showing chain structure and interchain disulfide bonds. The fragments produced by various treatments are also indicated.

portions of a heavy and a light chain and that Fc contains only heavy-chain components. Based on these results, Porter proposed a prototype structure for IgG, which has since been confirmed (Figure 5-3). According to this model, the IgG molecule consists of two identical H chains and two identical L chains, which are linked by disulfide bridges. The enzyme papain cleaves just above the interchain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these disulfide bonds, so that the two proteolytic enzymes generate different digestion products. Mercaptoethanol reduction and alkylation allow separation of the individual heavy and light chains.

Immunoglobulin Sequencing Studies

Initial attempts to determine the amino acid sequence of the Ig heavy and light chains were unsuccessful because sufficient amounts of homogeneous protein